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The ontogeny of leptin mRNA expression in growing broilers and its relationship to metabolic body weight

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Abstract

The polypeptide hormone leptin is produced by both adipose tissue and the liver and has been shown to induce satiety in chickens. In this study we have investigated the developmental regulation of leptin mRNA expression in growing broiler chickens. Leptin expression generally increases in all tissues from 1–12 weeks of age. In the subcutaneous fat depot there is an apparent pattern of increased leptin mRNA expression occurring at 2, 6, and 10 weeks post-hatch. This pattern was not evident in the other tissues surveyed and may relate to the cycle of loading and unloading of adipocytes with lipid. No consistent gender differences in leptin expression patterns were detected in the tissues surveyed, as is often observed in mammals. Positive correlations between metabolic body weight and adipose leptin expression levels were observed. Leptin expression by the liver was highly correlated with metabolic body weight from 1–6 weeks of age, and uncorrelated from 6–12 weeks of age. This pattern of increasing liver leptin expression with increasing body weight during the early rapid growth phase of the bird may be due to limited fat storage during this period, which is followed by rapid body fat accumulation from 6–12 weeks. The characterization and tissue specific distribution of leptin mRNA expression in the growing broiler indicate similar patterns of leptin production to that of growing mammals. Leptin may be involved in lipid flux through the adipocyte as well as the shift in lipid metabolism to increased storage during pre-puberty.

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1. Introduction

Leptin is the polypeptide hormone product of the obese (*ob*) gene and functions to regulate energy homeostasis [1]. Leptin is produced in the periphery and is involved in regulating food intake (FI), energy balance and reproduction in mammals and birds through a specific receptor in the hypothalamus [2,3,4]. Our interest lies in understanding the mechanism of FI regulation in meat-type chickens and leptin's potential role in regulating lipid metabolism. We, as well as others, have shown the presence of a leptin homolog in chickens and expression in both adipose and liver tissues [5,6,7]. This difference in tissue localization of leptin expression in mammals, such as rodents, may be due to avian lipid metabolism where the liver is the primary source of lipogenesis [8]. Previous studies have characterized the expression patterns of leptin in mammals through early growth and pre-puberty [9,10]. This study focuses on the expression pattern of leptin during the rapid growth phase of broiler production through the onset of maturity. During this process broilers undergo substantial growth until 6 weeks of age with relatively low accumulation of body fat whereas after 6 weeks of age fat deposition accelerates [11]. As an adipose derived hormone, leptin may play a role in this conversion of metabolism to increased lipid storage by regulating fatty acid homeostasis in the chicken [12].

2. Materials and methods

2.1. Animals

All animal studies were conducted with research protocols approved by the Beltsville Animal Care and Use Committee. Day-old male and female broiler chicks were purchased from Shaver Poultry Breeding Farms, Ltd., Cambridge, Ontario, Canada, and grown in brooder batteries until 3 weeks of age, at which time the birds were transferred to individual cages. Standard commercial starter and grower diets and water were available *ad libitum*. Tissues were collected at weekly intervals from 1–12 weeks of age from both male (n = 3) and female (n = 3) birds at the same time of day. Birds were sacrificed by cervical dislocation after which tissues were removed and snap frozen in liquid nitrogen within 5 minutes, for isolation of tissue specific RNAs.

2.2. QC-RT-PCR assay for leptin expression

Total RNA was isolated from collected tissues using the TRI-Reagent procedure (Life Technologies, Rockville, MD) and quantitated spectrophotometrically at 260 nm, with acceptable 260/280 ratios of >1.7. The quantitation of leptin mRNAs was performed as described previously [13]. Briefly, an RNA internal competitor that can be primed for cDNA synthesis and PCR amplification (234 bp) using the same primer set designed for the leptin target (261 bp) was constructed from a plasmid containing the full length leptin clone. Competitive RT-PCR amplifications (50 μL) included 5 μL of synthetic RNA (250, 50, 25, 5, and 2.5 amol/μL), random hexamer primers (instead of oligo dT) and for experimental

Table 1
Significance levels of associations between leptin expression levels and the variables of age, sex, and the interaction of age × sex

Tissue	Significance level		
	Age	Sex	Age × Sex
Liver	0.0001	0.304	0.6001
Fat Pad	0.0001	0.245	0.0028
SubQ Fat	0.0001	0.0001	0.0022

reactions 5 μg of total RNA. Additional RT reaction components and conditions were as described previously and were performed for each dilution of synthetic cRNA [13]. Calculation of the amount of unknown leptin mRNA present in each sample of total RNA isolated was performed by plotting the log of the ratio of cRNA product (234 bp) to the leptin mRNA product (261 bp) vs. log of the cRNA concentration added to each sample. The concentration of cRNA at which the log (cRNA/mRNA) = 0 indicates the concentration of leptin mRNA in the sample of total RNA.

2.3. Statistical analysis

Statistical analyses were performed using the GL model of SAS to perform ANOVA analyses of the relationship between leptin expression and age, sex, and age × sex. Regression of these data was performed using Microsoft Excel 98 software. Significant changes in leptin expression between individual samplings were performed using the Students t-test. Correlation coefficients between leptin expression levels and metabolic body weights were also determined using Excel.

3. Results

Leptin expression was quantitated for liver, omental and subcutaneous fat in both male and female broilers weekly from 1 to 12 weeks of age. Generally, leptin expression increased throughout post hatch growth and did not present any consistent sex differences in mRNA levels. Leptin expression in the liver ranged from 20 to 80 amol of mRNA per ug of total RNA analyzed. Expression increases throughout the early rapid growth phase until 6–7 weeks of age, after which it slightly declines as the birds reach maturity (Fig. 1A). ANOVA analysis indicated a significant association ($p < 0.01$) between liver leptin expression and age only. Following a similar pattern, leptin expression levels in the omental fat pad increased with age from 10 to 50 amol of mRNA per ug of total RNA (Fig. 1B). The ratio of liver expression levels to that of omental fat pad is approximately 2 to 1 at each time point sampled and is consistent with previous observations [5]. ANOVA analysis of omental fat pad leptin expression indicated a significant association ($p < 0.01$) with age as well as the combined effects of age × sex. The leptin expression levels in subcutaneous fat ranged from 10 to 50 amol of mRNA per ug of total RNA, similar to omental fat. Leptin expression in

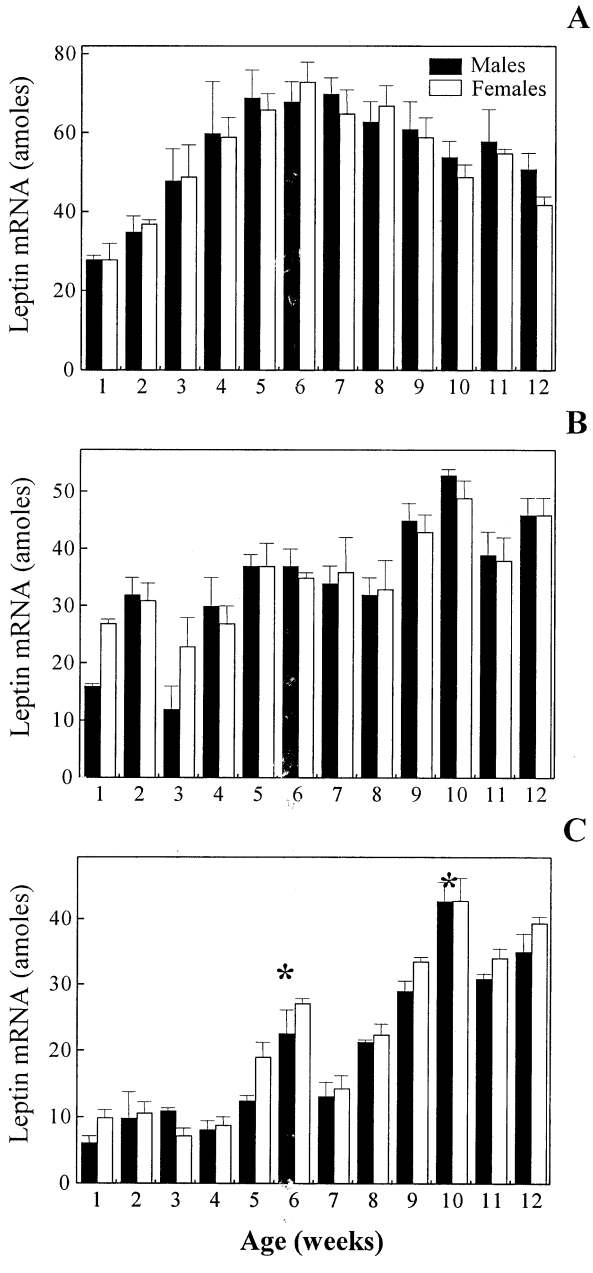

Fig. 1. Ontogeny of leptin expression levels in growing broiler chickens and the correlation to metabolic body weight. Leptin expression levels are presented as amoles of mRNA present per ug of total RNA isolated from liver (A), omental fat (B), and subcutaneous fat (C) at weekly interval from 1–12 weeks of age. Significant differences in leptin expression levels between a specific sampling time point, and preceding and subsequent sampling points ($p < 0.05$) are indicated by "*". Regression analysis indicated that the following were the simplest models that best approximated the observed data for each tissue ($p < 0.05$): liver, $y = 14.07 + 14.91x + -1.038x^2$, $R^2 = 0.79$; omental fat, $y = 19.23 + 2.79x + -0.046x^2$, $R^2 = 0.58$; subcutaneous fat, $y = 5.02 + 1.52x + 0.12x^2$, $R^2 = 0.80$.

subcutaneous fat appeared to increase in a pulsatile fashion with a periodicity occurring at 2, 6, and 10 weeks of age (Fig. 1C). ANOVA analysis indicated significant ($p < 0.01$) associations between age, sex, and age × sex with subcutaneous leptin expression over the duration of the study. The increases at 6 and 10 weeks of age were significantly different ($p < 0.05$) from measurements from the previous and following weeks.

Metabolic body weight was calculated by the formula [Metabolic body weight = (Body Weight)$^{0.75}$]. When plotted against leptin expression levels measured by QC-RT-PCR, metabolic body weight exhibited a positive correlation in both adipose tissues with similar coefficients (Fig. 2). In the liver, leptin expression levels are highly correlated to metabolic body weight only for the first 5–6 weeks of development ($R^2 = 0.94$). This close relationship dissolves beyond 6 weeks of post-hatch growth (Fig. 2).

4. Discussion

The results of this study present the first longitudinal study of leptin expression in avian species. The observation that leptin expression is dynamic throughout the growth and development of the chicken is similar to that reported to exist in mammals by many groups [9,10]. Leptin production in mammals is highly correlated with the fat depot size and increases with age through adolescence until puberty where there is an effect on sexual development and fertility particularly in females [14,15,16]. This relationship is reflected in the data collected in this study in growing chickens. Prior to sexual maturity there are very similar growth patterns between male and female broilers. Associations between leptin expression levels and the sex of the bird were only evident in measurements from the adipose depots. This difference in leptin expression as a function of sex or age × sex may reflect the influence of estrogen. We have previously described the repressive effects of estrogen on leptin expression in male broilers [5]. Unfortunately estrogen levels were not measured in this study and therefore will be the subject of future studies of its effects on leptin expression in the developing hen. The correlation of leptin expression with metabolic body weight throughout the study period in the adipose tissues surveyed is not surprising based on mammalian studies that have shown steady increases in leptin as the fat depot grows [17,18,19]. A novel observation that leptin expression in subcutaneous fat appears to undergo induction in a 4-week interval appearing at 2, 6, and 10 weeks of age was made in this study. This relationship may be in response to the cycling of adipocytes loading and unloading with lipid [20,21]. The adipocytes that express leptin in the chicken appear to only be those actively storing lipids as previously shown by immunohistochemistry [22]. The pattern observed may relate to the rate of lipid flux through the subcutaneous depot and thus the state of lipid/energy demand at specific points in development.

Correlations between leptin mRNA levels and metabolic body weights were observed for all three tissues surveyed. Positive correlations were observed between metabolic body weight and adipose leptin expression levels. This correlation closely mimics observations made in mammals during early development and likely reflects the increases in fat depot size. Similar experiments in chickens have shown that liver leptin expression is associated with fat depot size in lines of birds divergently selected for abdominal fat pad size [23]. Leptin

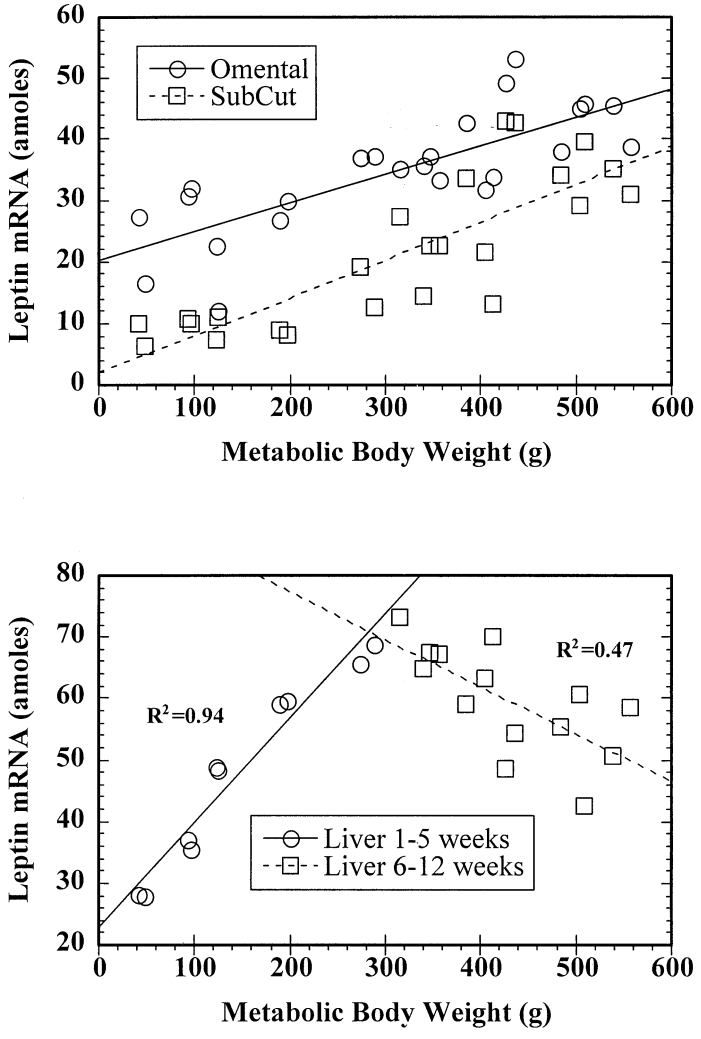

Fig. 2. Correlation between leptin expression levels and metabolic body weight in growing chickens. Leptin expression levels in adipose tissue when compared with metabolic body weight indicate a positive correlation $R^2 = 0.68–0.72$. In the liver leptin expression levels are highly correlated with metabolic body weight for the first 5–6 weeks of development ($R^2 = 0.94$) after which there is no apparent correlation.

expression by the liver in this study was highly correlated with metabolic body weight only from 1–6 weeks of age. At ages beyond 6 weeks the correlation fades. During this time frame is when the initial rapid growth rates of broilers muscle, bone, and metabolic organs slow and energy storage begins to occur. Liver leptin expression may be closely linked with increasing body weight during the early rapid growth phase of the bird and may reflect the essential lipogenesis performed by the liver during this period. Subsequent shifting of metabolism of the liver to increased lipogenesis and fat storage may disconnect this relationship and reflect the specific energy status of a specific nutritional state at the sampling times [19]. The role of hepatic leptin may be more dynamic in this later stage of pre-pubescent growth due to the

greater variability observed. Leptin may function as a signal or reporter of fat turnover in adipocytes as well as the shift in lipid metabolism at the end of the early rapid growth phase towards increased lipid storage.

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